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Original research paper

Acute toxicity and hepatoprotective effect of *Arum maculatum* on rat liver cirrhosis induced with thioacetamide

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ABSTRACT

Arum maculatum is a medicinal plant that has been employed in traditional medicine for treating liver diseases. The objective of the current study was to evaluate the hepatoprotective impacts of ethanolic extract of the *A. maculatum* leaves on cirrhosis induced by thioacetamide (TAA) in Sprague-Dawley rats. The rats were treated for two months with thioacetamide (TAA) administered intraperitoneally thrice weekly. Histopathological examination revealed severe liver damage in the thioacetamide control group, while the groups treated with *A. maculatum* showed reduced necrosis and fibrous tissue proliferation. Additionally, activities of antioxidant enzymes (SOD and CAT which were significantly reduced after TAA treatment), and the content of MDA (which was significantly higher in thioacetamide controls), were significantly improved after *A. maculatum* and silymarin treatments ($p < 0.05$). Furthermore, *A. maculatum* treatment led to the normalization of pro-inflammatory cytokines TNF-α and IL-6, and increased expression of the anti-inflammatory cytokine IL-10 ($p < 0.05$). Thus, *A. maculatum* leaves might have a hepatoprotective role in rat liver cirrhosis induced by TAA, along with antioxidant and anti-inflammatory effects.

Keywords: liver cirrhosis, *Arum maculatum* leaf, silymarin, thioacetamide, hepatoprotection

INTRODUCTION

Liver injury begins as inflammation and progresses to cirrhosis through an abnormal wound-healing process (1). Cirrhosis is a condition characterized by the gradual replacement of damaged liver cells by scar tissue (2). Liver cirrhosis can be triggered by diverse biological alterations in liver lobules, the accumulation of excessive fibrous tissue, and the development of small and large nodules within parenchymal layers, which are worsened by the formation of free radicals and a decrease in the activity of antioxidants (3). Additionally, hepatic damage is generated by a variety of factors, such as environmental pollutants, medications, alcoholism, obesity, and chronic diseases like diabetes.

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Thioacetamide (TAA) is a cost-effective organosulfur hepatotoxin that might lead to the development of liver cirrhosis *in vivo,* producing histological and biochemical alterations resembling those observed in cirrhosis in humans (1, 4–6), oxidative stress, and fibrosis through hepatotoxic metabolites (7, 8). It generates reactive oxygen species (ROS), reduces antioxidant enzymes like CAT, SOD and GSH (9), and promotes lipid peroxidation, necrosis, and chronic inflammation (10). TAA's active metabolite disrupts hepatocyte membranes, elevates pro-inflammatory cytokines (11, 12), and serves as a valuable model for studying liver diseases. Various studies in the literature have proven the beneficial effects of various medicinal herbs on hepatic protection against injury induced by TAA *in vivo* (13, 14).

Arum maculatum is a perennial herb with tuberous roots and arrowhead-shaped leaves (15), found in temperate and Mediterranean regions of Asia, Africa, and Europe (16). Traditionally used for medicinal and dietary purposes, it treats various disorders like inflammation, diabetes, gout, wounds, pain, and hypertension (17, 18). The plant contains diverse bioactive compounds, including polyphenols, alkaloids, saponins, and lectins, particularly concentrated in its tubers (16, 19, 20).

Despite the remarkable advancements in the modern medical system, it is still a dream to find a novel medication to cure liver cirrhosis. Consequently, traditional medical systems use a variety of medicinal herbs to treat liver diseases. Therefore, the goal of this study was to investigate the hepatoprotective impact of *Arum maculatum* leaf extract on liver cirrhosis in rats induced by TAA.

EXPERIMENTAL

Preparation of A. maculatum *leaf extract*

A. maculatum leaves were provided from a local market in the Ranya district within the Kurdistan region in northern Iraq. The identification of plants was conducted by a plant taxonomist affiliated with Raparin University. The leaves were air-dried at ambient temperature and then pulverized into a fine powder. To prepare the extract, 300 g of air-dried powdered leaves were suspended in 1 L of absolute ethanol in a beaker, and the mixture was stirred for 72 hours using a magnetic stirrer. After filtering the mixture through the Whatman filter paper (No. 1), the solution was centrifuged. The supernatants were transferred to a rotary evaporator for drying under reduced pressure. The yield of the crude extract was 5.9 % (*m*/*m*) and it was stored at 4 °C. The crude extract of *A. maculatum* leaves was then given orally to the rats at doses of 250 and 500 mg kg^{-1} body mass (5 mL kg^{-1} body mass) after being dissolved in 10 % Tween 20.

Chemical analysis of A. maculatum *leaves extract by GC-MS*

The GC-MS analysis of the ethanolic extract of *A. maculatum* leaves was conducted using a Shimadzu GCMS-QP2010SE system, under the following conditions: helium (99.999 % purity) was used as the carrier gas at a steady flow rate of 1 mL min^{-1}, with a sample injection volume of 1 μL and a split ratio of 1:1. The injector temperature was fixed at 280 °C, and the ion source was held at 230 °C. The oven temperature started at 60 °C

(held constant for 1 minute), then increased by 10 °C per minute to 240 °C, followed by 5 °C per minute to 300 °C, where it was held for 9 minutes. The total run time was 29.90 min, and mass spectra were obtained at 70 eV with a 0.5-second scan interval and the data was evaluated using total ion count (TIC) for both identification and quantification of the compounds. The spectra of the components were matched against a database of known spectra in the GC-MS library using the NIST and Wiley mass spectra library sources. The relative percentage of each component was estimated by comparing its average peak area to the total peak area. Peak area measurements and data processing were performed using Turbo-Mass-OCPTVS-Demo SPL software (Perkin Elmer, USA).

Thioacetamide solution

Thioacetamide was obtained from Sigma Aldrich (USA, purity 99 %) and was dissolved in 10 % Tween 20. The rats were given 200 mg kg^{-1} of TAA thrice per week for two months through an intraperitoneal injection (21).

Silymarin solution

Silymarin with a purity of 95 % was acquired from Sigma Aldrich. It was utilized as a control drug after being dissolved in a 10 % Tween 20 and 50 mg kg^{-1} administered orally to rats (22).

Animals

Throughout the experimental period, 66 healthy adult Sprague-Dawley rats (180–200 g), males and females, were housed individually in wire-mesh cages to prevent coprophagia and provided with standard pellet feed and water *ad libitum*. For the hepatoprotective activity study, 30 male rats were randomly divided into five groups (6 rats per group). The remaining 36 rats were divided into three groups (12 animals per group) for acute toxicity testing.

The animals were purchased from the Experimental House (Ethical No. Biology/22/10/2023/MAA, R), College of Science, Cihan University, Iraq.

Acute toxicity testing of A. maculatum *leave extract*

A total of 36 Sprague-Dawley rats (18 males and 18 females), were divided into three clusters (12 rats per group) and given (2 g kg^{-1} and 5 g kg^{-1}) of *A. maculatum* leave extract as well as a vehicle (10 % Tween 20). The rats were fasted overnight but were given excess water before receiving treatment. They were monitored for 30 minutes, as well as at 1, 2, 3, and 24 hours, to check for any signs of toxicity or mortality. After fasting overnight on day 14, the rats were sacrificed on day 15 under general anesthesia, consisting of ketamine (30 mg kg^{-1}, 100 mg mL^{-1}) and xylazine (3 mg kg^{-1}, 100 mg mL^{-1}).

Blood samples collected from the sacrificed rats were placed in gel-activated tubes and centrifuged at 2500 rpm for 15 minutes. The separated serum was used to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), as well as albumin, total bilirubin and total protein levels, and kidney function

parameters (urea and creatinine), and levels of sodium, potassium, and chloride ions. The liver and kidney organs of the sacrificed rats were dissected for histological analysis.

Hepatoprotective activity of A. maculatum *leaves extract*

Thirty Sprague-Dawley rats were categorized into five groups: group 1 (healthy control group) – received orally 10 % Tween 20 daily for two months, along with intraperitoneal injection of sterile distilled water three times per week; group 2 (TAA control group) – given 10 % Tween 20 and intraperitoneal injections of 200 mg kg^{-1} TAA three times per week; group 3 (positive control) – the rats received daily oral doses of silymarin (50 mg kg^{-1}) and intraperitoneal injections of thioacetamide (200 mg kg^{-1}) three times per week; groups 4 and 5 (*A. maculatum* fed groups) – rats were given *A. maculatum* leaves extract at low dose (LD) of 250 mg kg^{-1} and high dose (HD) of 500 mg kg^{-1} per day, resp., along with 200 mg kg^{-1} of TAA through intraperitoneal injection three times per week (23, 24). The rats were treated for two months. After two months and overnight fasting, the rats were sacrificed under general anesthesia.

Gross morphology of liver. – The livers were separated, washed with cold normal saline, and then placed on filter paper to examine for weight changes and obvious gross morphological characteristics to calculate liver index:

$$\text{liver index}(\%) = \frac{\text{liver mass}}{\text{body mass}} \times 100$$

Liver tissue histopathology. – Slices of hepatic tissue were fixed overnight in freshly prepared 10 % phosphate-buffered formalin (PBF). After trimming, the small tissue pieces were placed in cassettes for re-fixing in PBF and then embedded in paraffin using a mechanical tissue processing machine. Then, liver sections, 5 μm thick, were stained with hematoxylin and eosin (H&E) and Masson trichrome (25). Histopathological changes were examined using a 20× magnification Nikon microscope (Y-THS, Japan), and photos of characteristic areas were captured.

Endogenous antioxidant enzymes

Liver tissue samples were obtained from both lobes. After rinsing the samples with cold PBS, a Teflon tissue homogenizer (Polytron, Heidolph RZR 1, Germany) was used to homogenize the liver tissues. The homogenized samples were centrifuged for fifteen minutes at 3500 rpm to remove cell debris. The supernatants were used to measure the activity of CAT and SOD enzymes using a Cayman Chemical Company (USA) kit. The degree of oxidative stress was also evaluated using malondialdehyde (MDA) *via* a producer protocol (TBARS, Cayman Chemical Company).

Inflammatory cytokines

An ELISA kit (Cusabio Biotech Co., China) was used to detect the pro-inflammatory cytokines IL-6 and TNF-α, as well as the anti-inflammatory cytokine IL-10. Briefly, liver tissue homogenate was centrifuged for 15 minutes at 3000×*g*, and the resulting supernatant was analyzed using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

The mean ± standard error was used to present the results. Analysis of variance (ANOVA) was used to find the significant differences between group means, and Tukey's test was subsequently used to compare the doses. $p < 0.05$ value is set as the level of significance. A version of GraphPad Prism 9.5.1 was used to generate the figures and perform statistical analysis.

RESULTS AND DISCUSSION

Chemical composition of A. maculatum *leaf ethanolic extract*

The GC-MS chromatogram of the ethanolic extract of *A. maculatum* leaves (Fig. 1) revealed the presence of bioactive compounds identified by comparing mass spectra with the NIST library (Table I). The most prevalent compounds are methyl linolenate (40.15 %), ethyl linolenate (17.40 %), ethyl palmitate (14.79 %), gamma sitosterol (5.57 %), lavandulyl acetate (3.40 %) and vitamin E (α-tocopherol) (1.70 %).

Predominant components, methyl linolenate (47.15 %) and ethyl linolenate (17.40 %) show different biological activities including antioxidant, hepatoprotective, anti-inflammatory, cancer preventive, hypocholesterolemic, cardiovascular protective, antibacterial and antiarthritic properties (26–29). Ethyl palmitate has demonstrated notable biological activities such as anti-inflammatory and antimicrobials (30). Gamma sitosterol shows anti-hyperlipidemic, antidiabetic, anticancer, anti-inflammatory, and cytotoxic properties (31,

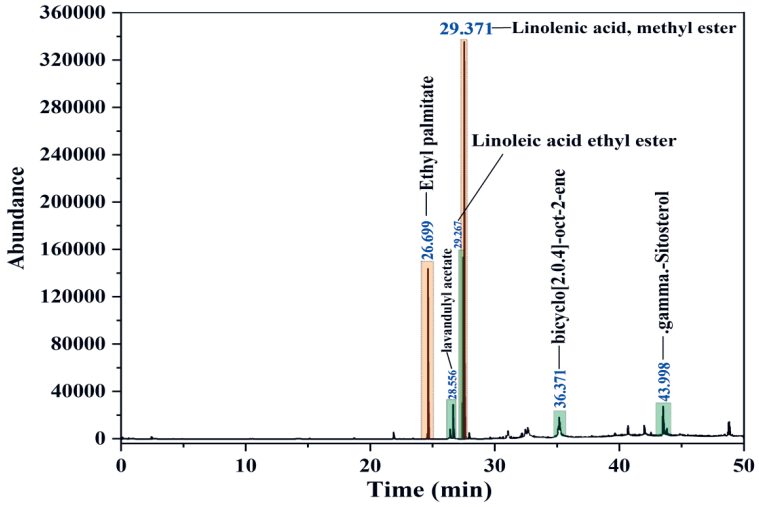

Fig. 1. GC-MS chromatogram of an ethanolic extract of *A. maculatum* leaves. The most abundant identified compounds are: ethyl palmitate, lavandulyl acetate, methyl linolenate, ethyl linolenate, bicyclo[4.2.0]-oct-2-ene, gamma sitosterol (for more details see Table I and Supplementary materials).

Table I. The most abundant/prominent compounds detected by GC-MS in ethanolic extract of A. maculatum *leaves*

Retention time (min)	Peak area (%)	Name of compounds	*m*/*z* value	Molecular formula
26.69	14.79	ethyl palmitate	284.0	$C_{18}H_{36}O_2$
28.55	3.40	lavandulyl acetate	138.0	$C_{12}H_{20}O_2$
29.26	17.40	ethyl linolenate	308.0	$C_{20}H_{36}O_2$
29.37	40.15	methyl linolenate	292.0	$C_{19}H_{32}O_2$
36.37	7.43	bicyclo[4.2.0]-oct-2-ene	108.0	C_8H_{12}
41.39	1.70	vitamin E (α-tocopherol)	–	$C_{29}H_{50}O_2$
43.99	5.57	gamma-sitosterol	414.0	$C_{29}H_{50}O$

32). Also, vitamin E (α-tocopherol) was identified in *A. maculatum* plant extract with marked antioxidant and anti-inflammatory characteristics (33). It plays important roles in skin integrity, gene regulation, wound healing, immune modulation, neuroprotection, and management of chronic diseases such as arthritis and diabetes (34).

Acute toxicity

Rats were observed for a period of two weeks after being treated with *A. maculatum* at doses of 2 and 5 g kg^{-1}. During the experiment, the rats stayed active and exhibited no changes in behavior, body mass, or gross. Throughout the 14-day trial, the rats showed no indications of toxicity or mortality at both dosages. Normal liver and kidney biochemical parameters are shown in Tables II and III with no significant change. Also, histopathological analyses of the liver and kidney demonstrated no significant differences between the *A. maculatum*-treated rats and the normal control group (Fig. 2). According to these results the *A. maculatum* leaf extract is considered to be safe at both doses.

Table II. Acute toxicity: Impact of A. maculatum *leaves extract on hepatic markers in rats*

Animal group	AST (IU L^{-1})[a]	ALT (IU L^{-1})[a]	ALP (IU L^{-1})[a]	Total protein (g L^{-1})[a]	Albumin (g L^{-1})[a]	Total bilirubin (μmol L^{-1})[a]
Normal control (10 % Tween 20)	55.83 ± 0.94	37.83 ± 0.94	78.00 ± 1.06	75.50 ± 1.11	24.16 ± 0.94	1.32 ± 0.009
A. maculatum (2 g kg^{-1})	60.33 ± 1.33	42.16 ± 0.94	71.16 ± 0.94	66.16 ± 1.49	21.16 ± 0.94	1.41 ± 0.009
A. maculatum (5 g kg^{-1})	58.00 ± 1.06	35.16 ± 0.94	75.66 ± 1.05	71.66 ± 1.05	25.83 ± 0.94	1.26 ± 0.009

ALT – alanine aminotransferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase

[a] Mean ± SEM (n = 6 rats per group).

Table III. Acute toxicity: Impact of A. maculatum *leaves extract on kidney function tests in rats*

Animal group	Sodium (mmol L^{-1})[a]	Potassium (mmol L^{-1})[a]	Urea (mmol L^{-1})[a]	Creatinine (μmol L^{-1})[a]	Chloride (mmol L^{-1})[a]
Normal control (10 % Tween 20)	141.36 ± 1.25	5.33 ± 0.09	4.76 ± 0.07	42.62 ± 1.15	106.66 ± 1.70
A. maculatum (2 g kg^{-1})	143.37 ± 1.27	5.26 ± 0.07	5.09 ± 0.03	38.63 ± 0.95	111.16 ± 1.35
A. maculatum (5 g kg^{-1})	138.55 ± 1.43	5.52 ± 0.09	4.78 ± 0.09	44.34 ± 0.94	104.33 ± 1.94

[a] Mean ± SEM (n = 6 rats per group).

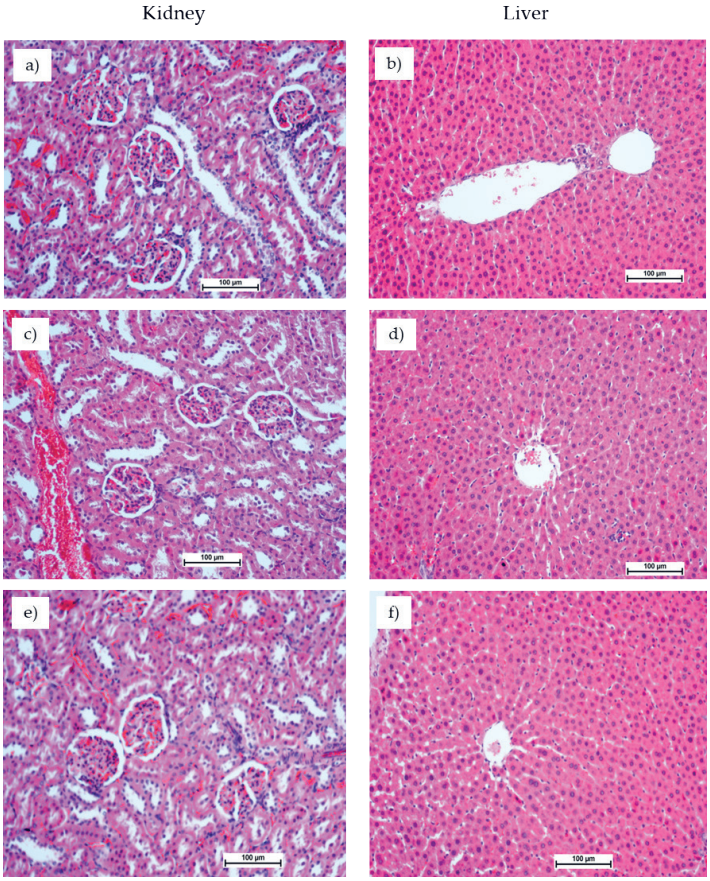

Fig. 2. Histological evidence of *A. maculatum* leaf extract influence on kidney and liver during the evaluation of acute toxicity: a) and b) rats were given 5 mL kg^{-1} of 10 % Tween 20 vehicle; c) and d) rats were given 2 g kg^{-1} of *A. maculatum*; e) and f) rats were given 5 g kg^{-1} of *A. maculatum* leaf extract.

Liver mass, liver index, and body mass

Throughout the trial, TAA's toxicity may have induced the increase in liver mass and decrease in body mass. Rats in the TAA control group showed a considerable decrease in body mass and an increase in liver mass (hepatomegaly) compared to the normal group. However, rats treated either with *A. maculatum* leaf extract or silymarin showed gains in body mass and a decrease in liver mass (Table IV). In line with the findings of our study, several researchers using different plant extracts have reported a reduction in liver mass or body mass ratios compared to the hepatotoxic group (8).

Table IV. Impact of A. maculatum *leaves ethanolic extract on body mass, liver mass, and liver index*

Animal group	Body mass (g)[a]	Liver mass (g)[a]	Liver index (LM/BM, %)[a]
Normal control (10 % Tween 20)	250.33 ± 1.52[c]	6.48 ± 0.09[c]	2.6 ± 0.03[c]
TAA + 10 % Tween 20	202.83 ± 3.19[b]	11.38 ± 0.09[b]	5.6 ± 0.1[b]
TAA + silymarin (50 mg kg^{-1})	261.50 ± 1.94[c]	7.46 ± 0.08[c]	2.9 ± 0.04[c]
TAA + *A. maculatum* (250 mg kg^{-1})	237.10 ± 1.46[c]	9.93 ± 0.14[c]	4.2 ± 0.04[c]
TAA + *A. maculatum* (500 mg kg^{-1})	259.50 ± 1.72[c]	8.73 ± 0.06[c]	3.4 ± 0.01[c]

LM – liver mass, BM – body mass

[a] Mean ± SEM (n = 6 rats per group).

Statistically significant difference between means with distinct superscripts within a column: [b] *versus* normal control $p < 0.05$, [c] *versus* TAA control $p < 0.05$.

Liver biomarkers

The alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin levels were substantially higher in the thioacetamide-treated group than in the silymarin or *A. maculatum* group because tissue injury causes liver enzymes to rise. These biomarkers rise in the cirrhotic group indicating cellular leakage and abnormal functioning of the liver cell membrane. In the rat groups treated with the *A. maculatum* extract, the ALP, AST, ALT, and total bilirubin levels markedly decreased. These findings suggested that the hepatic tissue damage caused by TAA treatment has been partly ameliorated and that the liver cell membrane has stabilized. Several researchers have associated these markers with studies on TAA-induced hepatotoxicity (14, 35).

Also, in this research, albumin and total protein concentrations were utilized for evaluating liver function because the synthesis of proteins, particularly albumin, occurs mainly in the liver. The total protein and albumin were considerably reduced in the thioacetamide-only group compared to rats treated with silymarin or *A. maculatum* groups, exhibiting acute hepatocellular injury. The findings are shown in Table IV. The elevating level of albumin after treatment with the *A. maculatum* extract compared with the values of TAA groups imply that the plant extract contributed to preventing a decrease in albumin

Table V. Impact of A. maculatum *leaves on liver biomarkers after TAA-induced hepatotoxicity in rats*

Animal group	ALP (IU L^{-1})[a]	AST (IU L^{-1})[a]	ALT (IU L^{-1})[a]	Total protein (g L^{-1})[a]	Albumin (g L^{-1})[a]	Total bilirubin (μmol L^{-1})[a]
Normal control (10 % Tween 20)	71.35 ± 0.47[c]	60.03 ± 0.75[c]	32.30 ± 0.53[c]	62.11 ± 0.58[c]	21.32 ± 0.30[c]	1.15 ± 0.01[c]
TAA + 10 % Tween 20	222.01 ± 2.32[b]	210.44 ± 1.61[b]	152.14 ± 2.45[b]	42.54 ± 0.76[b]	12.09 ± 0.33[b]	4.50 ± 0.16[b]
TAA + silymarin (reference drug control) (50 mg kg^{-1})	75.66 ± 1.29[c]	62.73 ± 0.53[c]	32.46 ± 0.53[c]	61.62 ± 0.51[c]	19.97 ± 0.50[c]	1.16 ± 0.01[c]
TAA + *A. maculatum* (250 mg kg^{-1})	82.74 ± 0.73[c]	84.83 ± 1.06[c]	75.58 ± 0.90[c]	56.48 ± 0.98[c]	16.99 ± 0.46[c]	2.02 ± 0.05[c]
TAA + *A. maculatum* (500 mg kg^{-1})	78.76 ± 1.45[c]	66.23 ± 1.46[c]	36.03 ± 1.13[c]	58.88 ± 1.36[c]	18.04 ± 0.92[c]	1.22 ± 0.03[c]

ALP – alkaline phosphatase (ALP), ALT – alanine aminotransferase, AST – aspartate aminotransferase

[a] Mean ± SEM (n = 6 rats per group).

Statistically significant difference between means with distinct superscripts within a column: [b] *versus* normal control $p < 0.05$, [c] *versus* TAA control $p < 0.05$.

level either by resynthesizing proteins and maintaining the endoplasmic reticulum or by using antioxidant chemicals to neutralize ROS. Accordingly, several researchers demonstrated that rats treated with silymarin or various plant extracts restored albumin and protein levels to normal (36, 37).

Histopathological analysis

Hematoxylin and eosin were used to stain the liver tissues to observe histological alterations. The findings shown in Fig. 3a (H&E) of the normal control group liver tissue showed characteristic hepatocyte architecture, an intact cytoplasm, and prominent nucleoli and nuclei with different regular plates of liver cells divided by central veins and sinusoidal capillaries, while the TAA control group (Fig. 3b, H&E) revealed significant liver damage, as proven by the presence of necrotic tissue, fibrosis, inflammatory cells, mononuclear infiltrate, and collagen deposition, as well as the development of bile ducts, hepatic cells, and cytoplasmic vacuolation. Moreover, liver architecture partially disappeared in the TAA group. Hepatocytes with deterioration between lobules were observed, and the vein in the centrilobular area looked slightly dilated. The sinusoids exhibited no abnormalities, and the periportal region showed signs of inflammation-induced mild lymphocyte infiltration. These various symptoms possibly occur because of the damaging effects of TAA, namely, acetaldehyde, which may be destructive to hepatocyte membranes.

Meanwhile, Figs. 3c,d,e (H&E) of the silymarin + TAA, low dose *A. maculatum* extract (250 mg kg^{-1}) + TAA, and high dose *A. maculatum* extract (500 mg kg^{-1}) + TAA groups showed a reduction in the number of inflammatory cells, and the liver architecture was

restored compared to the cirrhotic groups. A few hepatocytes displayed evidence of regeneration and a small amount of lymphocyte infiltration. This implies that the dose of 500 mg kg^{-1} of *A. maculatum* may considerably preserve liver cells from the harmful effects of TAA, which may be connected to the extract's bioactive components (16, 20, 38). These results confirmed the protective effects of *A. maculatum* and silymarin against liver damage caused by TAA.

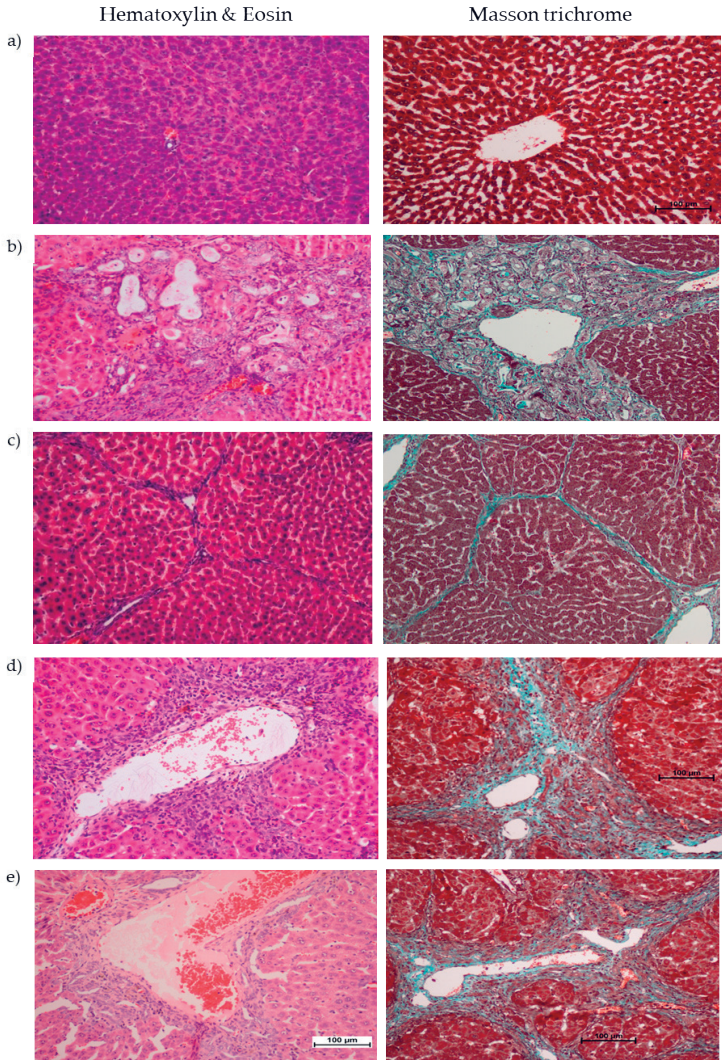

Fig. 3. Hematoxylin and eosin (H&E) and Masson trichrome stains in histopathological examination of liver tissue sections: a) normal control group; b) 200 mg kg^{-1} TAA-treated group; c) 50 mg kg^{-1} silymarin group; d) rats treated with low dose of *A. maculatum* leaf extract (250 mg kg^{-1}); e) rats treated with high dose of *A. maculatum* (500 mg kg^{-1}).

Moreover, the degree of fibrosis in the liver samples was determined by Masson's trichrome staining (MT) (Fig. 3). The liver samples from the normal group (Fig. 3a) showed no evidence of collagen accumulation in the cirrhotic livers. In Fig. 3b, an obstructed central vein encircled by densely accumulated collagen fibers was observed, revealing a significant degree of fibrosis after TAA-induced liver cirrhosis. In the silymarin (positive control) group (Fig. 3c), livers had extremely low levels of fibrosis, as demonstrated by the very low deposition of collagen fibers. Animals administered (250 mg kg^{-1}) and (500 mg kg^{-1}) of *A. maculatum* extract (Figs. 3d,e) exhibited a notable decrease in the amount of collagen deposition surrounding the central vein. The rats treated with *A. maculatum* showed a gradual decline in the degree of fibrosis in liver sections. According to Masson's trichrome, a decrease in collagen deposition in tissue sections of the *A. maculatum* leaf extract groups contrasted with the TAA group. A number of studies that employed various medicinal herb extracts have shown a reduction in collagen fibers against cirrhotic liver tissue caused by TAA, which is consistent with the findings of the current investigation (36).

Effect of A. maculatum *on antioxidant enzymes*

The cirrhotic rats administered TAA exhibited lower levels of antioxidant enzymes (CAT and SOD) compared to the normal control group (Fig. 4). These results demonstrated

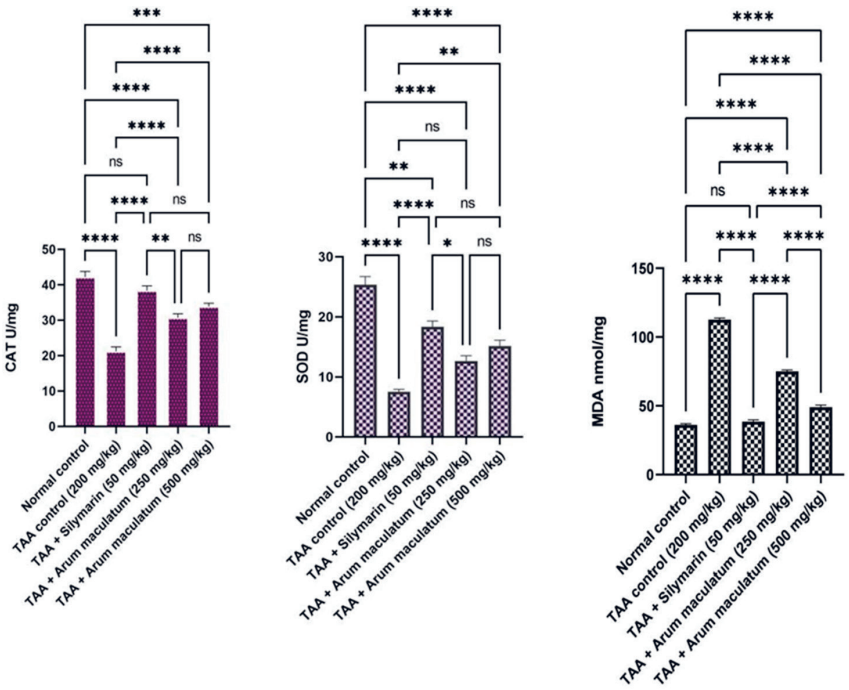

Fig. 4. Influence of *A. maculatum* leaf extract on the levels of CAT, SOD and MDA in rat liver homogenates. TAA control group: 200 mg kg^{-1}, treatment with *A. maculatum*: 250 mg kg^{-1} and 500 mg kg^{-1}, treatment with silymarin: 50 mg kg^{-1}). Data displayed as mean ± SEM (n = 6 rats per group). Statistical significance at: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns – not significant.

the occurrence of damage in cirrhotic liver cells (hepatocytes), while experimental groups treated with *A. maculatum* extract showed a considerable restoration of CAT and SOD. Rats given *A. maculatum* extract significantly ($p < 0.05$) increased SOD and CAT levels in comparison to those in the hepatotoxic group. *A. maculatum* extract and silymarin might considerably accelerate the healing of liver damage.

Malondialdehyde (MDA) is generated from the peroxidation of unsaturated fatty acids and is an oxidative stress marker: a higher MDA level indicates oxidative damage in hepatic injury (39). Apparently, the reduced levels of liver antioxidant enzymes CAT and SOD in the TAA-treated group could help to justify the elevated MDA levels (40). The mean levels of MDA in liver homogenates from the TAA control group were higher than those in the normal, silymarin, LD, and HD *A. maculatum*-treated groups (Fig. 4). The current investigation demonstrated that lipid peroxidation was reduced ($p < 0.05$) by *A. maculatum* extracts at both doses of 250 mg kg^{-1} and 500 mg kg^{-1}. Additionally, research performed by Al-Shmgani *et al*. (41) suggested that hydrogen-donating substances found in *A. maculatum* leaf extracts have the capability to remove free radicals.

Impact of A. maculatum *leaf extract on the level of inflammatory cytokines*

IL-6 and TNF-α are inflammatory mediators that impact the functioning of organ homeostasis and cause hepatic damage in experimental models of liver injury. A number

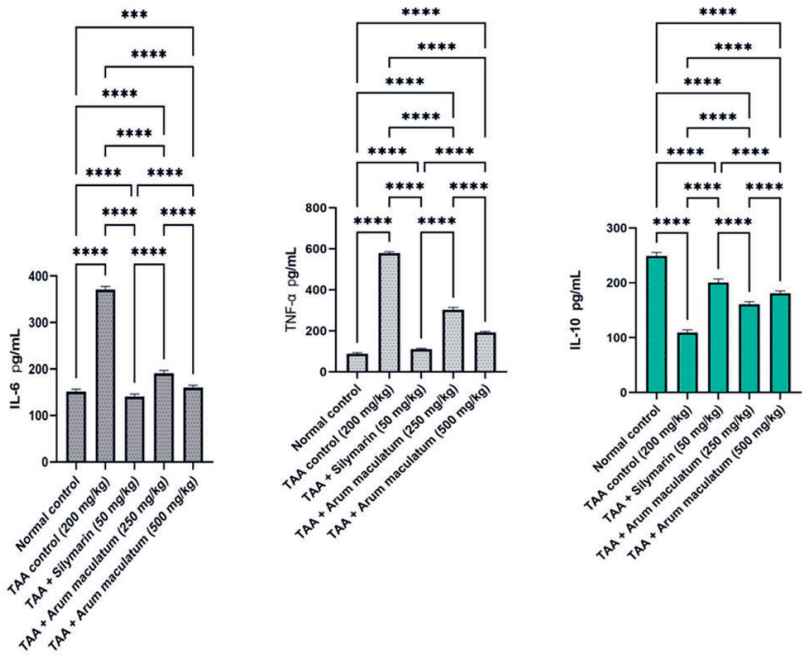

Fig. 5. Impact of *A. maculatum* leaf extract on IL-6, TNF-α, and IL-10 in rats with liver cirrhosis induced by TAA. TAA control group: 200 mg kg^{-1}, treatment with *A. maculatum*: 250 mg kg^{-1} and 500 mg kg^{-1}, treatment with silymarin: 50 mg kg^{-1}. Data expressed as mean ± SEM ($n = 6$ rats per group). Statistically significant difference: ***$p < 0.001$, ****$p < 0.0001$.

of investigations have shown that IL-10 has immunomodulatory, antifibrotic, and anti-inflammatory properties. It can also control and reduce pro-inflammatory cytokine expression (42).

The serum inflammatory cytokines exhibited that the levels of IL-6 and TNF-α in liver homogenates from the TAA control group were significantly ($p < 0.05$) higher whereas level of IL-10 of the TAA control rats was significantly ($p < 0.05$) lower compared to the normal control (Fig. 5). When compared to the TAA group, treatment with *A. maculatum* leaf extract and silymarin exhibited a significant ($p < 0.05$) decrease of IL-6 and TNF-α and elevated IL-10 levels. In regard to increased IL-10 release by the use of *A. maculatum* leaf extract is consistent with the outcomes of other studies (43). According to these findings, inflammatory cytokine upregulation is linked to cirrhosis and might be partly ameliorated by *A. maculatum* leaf extract.

CONCLUSIONS

The acute toxicity testing demonstrated that the ethanolic extract of *A. maculatum* leaf was safe, with no toxic effects on histology and biochemistry parameters of the liver and kidney. According to biochemical and histological assessments, this extract might even display possible hepatoprotective potential against TAA-induced cirrhosis in rat liver. The hepatoprotective activity of the *A. maculatum* leaf extract may also be associated with its inducing actions on the CAT and SOD antioxidant enzymes and its inhibitory potential on the level of MDA. *A. maculatum* leaf extract decreases the levels of pro-inflammatory cytokines TNF-α and IL-6 levels and increases the anti-inflammatory cytokine IL-10. Accordingly, *A. maculatum* leaf extract could possibly serve as an effective herbal product for preventing chemically-induced hepatic damage, likely due to its ability to inhibit hepatocyte proliferation and reduce oxidative stress and lipid peroxidation.

The identification of bioactive compounds in *A. maculatum* extract was done through GC-MS analysis. Among the identified compounds, methyl ester of linolenic acid, ethyl ester of linoleic acid, ethyl ester of palmitic acid, and gamma sitosterol might have contributed to the observed effects.

The data supporting the results are available upon request.

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